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Factors Affecting the Content of **ASCORBIC ACID IN TOMATOES**

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Factors Affecting the Content of Ascorbic Acid in Tomatoes

HUSSEIN H. HASSAN and J. P. McCOLLUM^a

THE TOMATO (*Lycopersicon esculentum*) is important in nutrition primarily because of its ascorbic-acid (vitamin C) content. Efforts to increase the ascorbic-acid content of commercial varieties by breeding have not been too successful, even with genetically superior strains available. The slow progress in increasing the anti-scorbutic value of tomatoes has been due primarily to the difficulty of comparing strains because of errors in sampling. Such errors are indicated by the highly variable and conflicting data in the literature.

The purpose of this study is to evaluate some of the factors affecting the ascorbic-acid content of tomato fruits in an effort to reduce sampling errors and increase the accuracy of strain comparisons.

REVIEW OF LITERATURE

The literature dealing with the ascorbic-acid content of tomatoes has been well reviewed by Maclinn and Fellers,^{23*} Hamner and Maynard,¹² and Åberg.¹ For this reason only representative investigations are cited here.

Maclinn, Fellers, and Buck²² reported on the ascorbic-acid content of 98 tomato varieties tested in Massachusetts. They found variations among the different varieties ranging from $44 \pm .03$ to $13 \pm .03$ milligrams per 100 grams of fresh weight. They also reported great variations among samples of the same variety. For example, they found values varying from 19 to 50 milligrams per 100 grams fresh weight for the Bonny Best variety, and from 19 to 48 milligrams for John Baer. They found Marglobe 28 percent higher in ascorbic acid than Prichard. On the other hand, Tripp, Satterfield, and Holmes,⁴⁰ in a varietal test in North Carolina, reported Prichard high in ascorbic

* Superior figures refer to literature cited on pages 22 to 24.

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acid and as containing 27 percent more than Marglobe. Hamner, Bernstein, and Maynard¹⁴ showed wide variations in the ascorbic-acid content of tomato varieties grown at different locations. Fruits of Marglobe varied from 14.4 ± 2.12 grams of ascorbic acid to 30.6 ± 2.04 grams with location; Rutgers, from 8.4 ± 0.50 to 19.7 ± 1.17 grams; and Prichard, from 10.7 ± 0.88 to 29.0 ± 1.29 grams.

Although a number of investigators^{5, 8, 27} have reported definite varietal differences in the ascorbic-acid content of tomatoes, Currence,⁷ with samples obtained from a replicated field experiment, showed that differences between varieties were indefinite and difficult to demonstrate statistically.

Breeding for tomato varieties with high ascorbic-acid content, Shivrina³⁶ reported values as high as 43 milligrams per 100 grams fresh weight for cultivated varieties and as high as 80 milligrams in some wild varieties. In 1938 Biryukov³ crossed varieties having high and low potencies and found the first generation to have an ascorbic-acid content close to that of the parent with the higher values. In 1942 Reynard and Kanapaux³² reported that single determinations on each of 166 second-generation plants from a Red Current tomato \times Marglobe cross showed that the ascorbic-acid content in the F_2 generation ranged from about 9 to 42 milligrams per 100 grams fresh weight, while the parental lines had 42 and 16 milligrams respectively.

The influence of chromosome number on the vitamin-C content of tomato fruits has been the subject of considerable discussion in the literature. In 1933 Key²⁰ reported that tomatoes of the same genetic constitution, whether of diploid (12-pair) or tetraploid (24-pair) plants contained equal quantities of ascorbic acid. Later in the same year Sansome and Zilva³⁴ reported that tomatoes from tetraploid plants contained twice as much ascorbic acid as tomatoes from diploid plants. In 1935 McHenry and Graham²⁶ compared tetraploid with diploid fruits and reported that the diploid fruits contained about 84 percent as much ascorbic acid as the tetraploid, a difference considerably less than that reported by Sansome and Zilva.³⁴ However, in another report published in 1936 Sansome and Zilva³⁵ stated that they were unable to record again such a great inequality between the ascorbic-acid contents of tetraploid and diploid forms as they had reported in 1933. They suggested that an unknown factor or factors must have been responsible for that inconsistency. McHenry and Graham²⁶ claimed that the differences reported between tetraploid and diploid tomatoes were due to fruit size, as tetraploid tomatoes are usually smaller than diploid tomatoes.

Considerable disagreement is found concerning the relation between fruit size and ascorbic-acid content. Maclinn, Fellers, and Buck,²² as well as other investigators,^{9, 23, 40} reported no correlation between ascorbic-acid content and fruit size within a strain or a variety of tomatoes. Hallsworth and Lewis¹⁰ found a rapid and highly significant increase of ascorbic acid with decreasing weight (correlation coefficient -0.94) for tomato fruits weighing less than 30 grams; whereas fruits weighing 30 grams or more gave a nonsignificant coefficient of -0.03 . Several investigators,^{5, 26, 32} however, found ascorbic acid to be negatively correlated with fruit size.

In 1937 Maclinn, Fellers, and Buck²² reported that degree of ripeness had no significant effect upon the vitamin-C content of fruits picked from six tomato varieties. Conversely, Lo Coco²¹ in 1945 reported that the ascorbic-acid content was highest near and before the ripening stage and lowest in green and overripe tomatoes.

Conflicting reports have been published on experiments dealing with the effect of soil fertility and plant nutrients. Hester and Kohman¹⁷ reported that tomatoes grown on Sassafras sandy loam soil were 44 percent higher in ascorbic acid than those on Edgemont stony loam. However, the tomatoes on the sandy loam were grown at Moorestown, New Jersey, and those on the stony loam at Elverson, Pennsylvania, and harvested one month later. Hester and Kohman¹⁸ reported that the application of potassium fertilizer to certain soils resulted in an increase in the yield and in the vitamin-C content of tomato fruits. They also reported similar effects for the application of manganese to soils deficient in this element.¹⁹ Hamner, Lyon, and Hamner¹¹ showed that the ascorbic-acid content of tomatoes was not appreciably affected by wide variations in the amounts of macronutrient elements supplied to the plant.

Murphy²⁹ reported that environmental agencies markedly influence the synthesis of vitamin C in tomatoes. She stated that the geographical situation is not a contributing factor except insofar as environmental conditions are consistently characteristic of that situation. Hamner, Lyon, and Hamner,¹¹ working with the Bonny Best variety, reported that the location where the crop is grown has an effect upon the ascorbic-acid content of the fruits. They found the ascorbic-acid content to be associated apparently with differences in the environment of the top of the plant but not with differences between soils at the several locations.

Of the various environmental factors that influence the accumulation of ascorbic acid in plants, light seems to have the greatest effect.^{28, 31} In British Columbia, Harris¹⁶ noticed that the warmer

districts, with plenty of sunshine, yield tomatoes with the highest vitamin-C content. Pepkowitz *et al*³⁰ found higher ascorbic acid in peas associated with the wider spacing, which allowed more light to the plants. McCollum²⁴ reported that fruits picked from defoliated tomato plants were remarkably higher in ascorbic acid than those from plants with normal foliage. He stated that this difference in the ascorbic-acid content was due to the greater exposure of fruits to sunlight when the tomato plants were defoliated.

Clow and Marlatt⁶ found that greenhouse tomatoes allowed to ripen on the vine were not quite so potent a source of vitamin C as field tomatoes ripened on the vine.

Brown and Moser⁴ reported that tomatoes from vines supported on poles were significantly higher in ascorbic acid than those picked at the same time from adjacent unsupported vines. Obviously the supported vines received more over-all light.

Currence,⁷ growing a number of tomato varieties in the greenhouse, found significant differences in ascorbic-acid content from week to week and noticed that such differences did not appear to be associated with percentage of sunshine.

Somers, Hamner, and Nelson³⁷ reported that the amount of light for 18 days prior to harvest is closely correlated with the ascorbic-acid content of tomatoes under field conditions. In a later and more extensive experiment, however, by Somers, Hamner, and Kelly,³⁹ these results were not substantiated. Hamner and Parks,¹³ working with turnip greens, noticed that light intensities just prior to harvest play the dominant role in determining ascorbic-acid level. Similar results have been reported by Åberg¹ for tomato leaves.

McCollum²⁵ reported that exposed field tomatoes were remarkably higher in ascorbic acid than the shaded fruits from the same variety. He also showed that the upper half of the tomato fruit which received more sunlight was higher in ascorbic acid than the lower half. He stated that uniformity to light exposure ought to be considered in selecting tomato samples representing different treatments. He suggested the use of samples composed of equal opposite sectors taken from 12 to 15 either shaded or unshaded fruits.

Hamner, Bernstein, and Maynard¹⁴ transferred staked tomato plants of the Bonny Best variety at different developmental stages from sunshine to shade, and others from shade to sunshine, and reported that shading during the period just prior to ripening of the fruits increased the ascorbic-acid content. The effectiveness of illumination on the fruit and foliage was not measured separately. They also reported an increase in the ascorbic-acid content of the fruits with

an increasing photoperiod in the control chambers. However, they reported only a 17-percent increase with a 16-hour day in comparison with an 8-hour day.

Hansen and Waldo¹⁵ reported that strawberries grown and ripened under reduced light intensity contained less ascorbic acid than those ripened on plants fully exposed to light. They also found that shading the entire plants resulted in a much greater reduction in ascorbic acid than did shading the berries only. Recently Robinson³³ showed evidence that the ascorbic-acid content of strawberry fruits was affected by the amount of illumination received by the leaves.

Veselkine *et al.*,⁴¹ kept some developing tomato fruits in double bags of black paper and compared the ascorbic-acid content of the bagged fruits with that of comparable light-exposed control fruits. Their data were inconclusive but indicated that the light-exposed fruits were higher in ascorbic acid.

MATERIALS AND METHODS

The experiments reported here were conducted at Urbana, Illinois, on both field and greenhouse tomatoes. Two varieties, Illinois T19 and Garden State, were used in the field experiments. Seedlings from both varieties were transplanted for the crops of 1948 and 1949 on May 21 and May 19 respectively. In 1949 two additional plantings were made on June 14 and July 11.

For the greenhouse experiments the Lloyd forcing variety was used. The plants were transplanted on February 16, 1949, in raised benches running east and west. The greenhouse was maintained at day temperatures between 70° and 75° F. The plants were pruned to a single stem and topped at about 5 feet after producing from four to six clusters.

Light was excluded from fruits at different stages of development by enclosing them in bags made from aluminum-covered paper.^a The bags were then closed from the bottom by means of two or more small clips in a way that permitted air exchange inside the bags.

Fruits for analyses were harvested between 11 and 12 o'clock in the morning and analyzed the same day. Unless otherwise specified 12 to 15 selected fruits were used for each sample. Equal opposite sectors were cut from each fruit, making a total of 200 to 250 grams for analysis. The whole fruit was used when analyzed individually. Each fruit was harvested for analysis when entirely red.

^a Secured from the Tested Papers of America, Inc., Chicago, Ill.

For ascorbic-acid analyses the samples were blended for two minutes in a Waring blender with one milliliter of 0.5-percent oxalic acid per gram of tomato. The mixture was then allowed to settle, and a portion was filtered through Whatman No. 12 filter paper. Duplicate 3-milliliter aliquots of the filtrate in 5 milliliters of 0.2-oxalic acid were titrated with 2,6-dichlorobenzenoneindophenol according to the method of Bessey and King.²

Total solids were determined by drying duplicate 20-gram samples of juice at 80° C. in a Brabender ventilated oven until a constant weight was reached.

EXPERIMENTS AND RESULTS

Variation in Ascorbic-Acid Content of Shaded and Exposed Fruits

In order to study sample variability, shaded and exposed fruits of Illinois T19 and Garden State varieties were analyzed individually for ascorbic-acid content. Fruits from the 1948 field crop were used.

Table 1. — Variability in Ascorbic-Acid Content of Exposed and Shaded Tomato Fruits

(Data are given in milligrams per 100 grams fresh fruit)

Number of fruits	Illinois T19		Garden State	
	Exposed	Shaded	Exposed	Shaded
30.....	37.2 ± 0.57	26.3 ± 0.47	33.6 ± 0.53	21.6 ± 0.62
15.....	37.8 ± 0.77	26.3 ± 0.50	32.6 ± 0.63	21.3 ± 0.96
15.....	36.6 ± 0.83	26.2 ± 0.82	34.5 ± 0.81	21.8 ± 0.83
10.....	37.5 ± 0.95	26.2 ± 0.65	32.5 ± 0.89	22.3 ± 1.14
10.....	38.7 ± 0.75	25.8 ± 0.75	33.3 ± 0.78	19.9 ± 0.83
10.....	35.3 ± 0.99	26.8 ± 1.05	34.9 ± 1.01	22.5 ± 1.14

Samples of 30 well-exposed fruits of each variety and 30 fruits well shaded by foliage were selected at random for analyses. The exposed and the shaded fruits of Illinois T19 were harvested on August 18 and 19, those of Garden State on August 20 and 21. Calculations were made of the mean, the standard error of the mean, and the coefficient of variation for each of the four 30-fruit groups. Similar calculations were made for subsamples of 15 and 10 fruits each.

The exposed fruits (Table 1) were remarkably higher in ascorbic-acid content than the shaded fruits. The differences amounted to 41.4 percent with Illinois T19 and 55.6 percent with Garden State. In ad-

dition, both the exposed and the shaded fruits of Illinois T19 were significantly higher in ascorbic acid than the respective exposed and shaded fruits of Garden State. The shaded fruits of Garden State were more variable than the exposed ones. The difference is especially evident if the coefficients of variations are compared.

Effect of Sunlight on Ascorbic-Acid Content of Fruits at Various Stages of Development

Since the exposure of fruits to sunlight increases the ascorbic-acid content, experiments were designed to determine the stage of fruit development at which sunlight is most effective. This was done by excluding light from fruits at various stages of development with aluminum-covered paper. Experiments were conducted on both field and greenhouse tomatoes. Date of bagging, date of harvesting, fruit weight and diameter, and ascorbic-acid content were recorded.

Light excluded at turning stage of fruit. On August 9, 1948, a number of exposed fruits of the Illinois T19 variety were bagged at the turning stage. A number of similar fruits were marked for controls. The fruits were harvested when ripe and analyzed for ascorbic-acid content. Thirty bagged and thirty control fruits were harvested between August 15 and 20 and analyzed individually for ascorbic-acid content. The average time required for ripening was 7 to 8 days with both the treated and the control fruits. The experiment was repeated with the Garden State variety. Fruits were bagged September 12 and harvested between September 18 and 24. The results of the analyses are shown in Table 2.

The values for the bagged fruits averaged slightly lower than those for the controls, but the difference was not significant. There was no

Table 2.—Effect of Excluding Light at Turning Stage on Ascorbic-Acid Content of Tomato Fruits

(Data are given in milligrams per 100 grams fresh fruit)

Treatment	Illinois T19 ^a		Garden State ^a	
	Ascorbic-acid content	L.S.D. at 5%	Ascorbic-acid content	L.S.D. at 5%
Fruits bagged.....	31.1 ± 0.52	27.0 ± 0.54
Fruits exposed.....	32.2 ± 0.49	1.43	27.6 ± 0.55	1.54

^a Average of 30 fruits.

noticeable effect of bagging on the time required for ripening, on diameter, or on weight of fruit.

Fruits bagged 25 days after flowering. Because of the discrepancy in the literature^{37, 38} regarding the relation of sunlight during the last few weeks before harvest to the ascorbic-acid content, experiments were made to study the effect of illumination on fruits in the late green stage. Flowers of the Illinois T19 variety were tagged in the field on August 1, 1948. Twenty-five days later some of the fruits from the tagged flowers were bagged, while others were left exposed to sunlight. As the treated and control fruits ripened between September 12 and October 1, they were harvested and analyzed for ascorbic acid. The experiment was repeated with the Garden State variety. Twenty-five-day-old fruits were bagged or marked as controls on September 11 and harvested between September 28 and October 13.

The average time from flowering to ripening in the control fruits of Illinois T19 was 50 days and in the bagged fruits, 51 days; while for Garden State the average was 50 days for each treatment. In each test the control fruits were significantly higher in ascorbic acid than the fruits from which light was excluded (Table 3). The amount in the bagged fruits was 17.6 percent lower in Illinois T19 and 14.0 percent lower in Garden State.

Light excluded at early green stage of fruit. Although the differences shown in Table 3 are highly significant, they are much less so than the differences between the shaded and exposed fruits in Table 1. The two groups of data indicated that illumination early in the development of tomato fruits might be a factor affecting their ultimate ascorbic-acid content. So further experiments with two varieties were conducted to study the effect of solar illumination during the early development of field-grown fruit.

Table 3. — Effect of Bagging Fruits 25 Days After Flowering on Ascorbic-Acid Content of Tomato Fruits

(Data are given in milligrams per 100 grams fresh fruit)

Treatment	Illinois T19 ^a			Garden State ^a		
	Ascorbic-acid content	Difference	L.S.D. at 1%	Ascorbic-acid content	Difference	L.S.D. at 1%
Fruits bagged.....	25.3 ± 0.60	23.4 ± 0.76
Fruits exposed.....	30.7 ± 0.64	5.4	2.34	27.2 ± 0.45	3.8	2.35

^a Average of 30 fruits.

Table 4. — Effect of Excluding Light at Early Green Stage on Ascorbic-Acid Content of Tomato Fruits

(Data are given in milligrams per 100 grams of fruit)

	Illinois T19 ^a		Garden State ^b	
	Ascorbic-acid content	L.S.D. ^c at 1%	Ascorbic-acid content	L.S.D. ^c at 1%
Fruits bagged for 10 days	25.0 ± 0.89	2.77	22.4 ± 0.67	2.58
Fruits bagged for 20 days	23.2 ± 0.58	2.11	20.1 ± 0.55	2.37
Fruits bagged until ripe (39 days)	16.8 ± 0.31	1.90	13.5 ± 0.61	2.47
Fruits exposed (<i>control</i>)	30.4 ± 0.54	26.8 ± 0.69

^a Average of 30 fruits. ^b Average of 25 fruits. ^c Compared with exposed fruits.

Since in preliminary tests it had been found that bagging the flowers prevented fruit development, this operation was delayed until the fruits were between 2 and 3 centimeters in diameter and about 15 days old. Fruits of Illinois T19 bagged July 14, 1949, were divided into three groups: the first group remained bagged for 10 days and was then exposed to light until ripe; the second remained covered for 20 days; and the third was left covered until ripe. Control fruits were exposed throughout their development. Thirty fruits each of all four light treatments were harvested between August 12 and September 2 and analyzed separately. The fruits of Garden State were bagged or marked August 4 and 25 from each of the four treatments harvested between August 27 and September 27.

In these tests highly significant differences occurred in the ascorbic-acid content of bagged compared with exposed fruits (Table 4), and the content declined as the period of light exclusion was lengthened. When light was excluded for 10, 20, and 39 days, the ascorbic-acid content was reduced 17.8, 23.7, and 44.7 percent respectively in fruits of Illinois T19, and 16.4, 25.0, and 49.6 in the Garden State fruits.

Leaves and fruit shaded. The effects of shading the leaves and fruits of tomato plants were shown in another experiment. Illinois T19 transplanted to the field on May 21, 1949, was used for the test. Treatments were as follows: (1) fruits were bagged approximately 15 days after flowering; (2) fruits were bagged as in (1) and the plants shaded with a double layer of cheesecloth on July 8; (3) plants were shaded as in the previous treatment but fruits were not bagged; (4) plants were exposed and fruits were shaded by foliage; (5) plants and fruits were exposed to direct sunlight. Two replications of eight-plant rows were used for each treatment. Samples of 12 to 15 fruits

Table 5.—Ascorbic-Acid Content and Total Solids of Tomato Fruits Under Different Light Treatments

Treatment	Ascorbic- acid content ^a	Total solids
	<i>mgs/100 grams^b</i>	<i>perct.^b</i>
1. Fruits bagged, plants unshaded.....	17.53	5.3
2. Fruits bagged, plants shaded with cheesecloth.....	17.35	4.7
3. Plants shaded with cheesecloth.....	25.08	4.8
4. Unshaded plants, shaded fruits.....	24.25	5.5
5. Unshaded plants, exposed fruits (<i>control</i>).....	33.52	5.6

^a L.S.D. (compared with control): at 5% level 1.90, 1% level 2.59.

^b Values are averages for six samples of 12 to 15 fruits each.

from each treatment were harvested August 6, 26, and September 11 and were analyzed for ascorbic acid and total solids.

No difference was found in the ascorbic-acid content of bagged fruits whether the plants were shaded or exposed (Table 5). Fruits shaded by cheesecloth which covered the entire plant had about the same ascorbic-acid content as fruits shaded by foliage only. The highest ascorbic-acid content was found in fruits exposed to direct sunlight on unshaded plants.

The total-solids content of the fruits was reduced slightly by shading the plants with cheesecloth, but shading the fruits had no significant effect. The cheesecloth reduced the illumination about 75 percent, as measured by a Weston illumination meter, Model 756.

Effect of Bagging on Fruit Development in the Field

Since bagging fruits at the green stage caused a remarkable decrease in ascorbic acid, other effects on the fruit, such as size, color, and date of ripening, were determined for individual fruits in the experiment reported in Table 4. In addition, temperatures of bagged and

Table 6.—Size of Tomato Fruits and Time Required for Maturity When Fruits Were Bagged at Early Green Stage and When Left Exposed

Average per fruit	Illinois T19 ^a		Garden State ^b	
	Bagged	Exposed	Bagged	Exposed
Weight in grams.....	149	143	110	112
Diameter in centimeters.....	6.8	6.9	6.3	6.3
Number of days between flowering and maturity.....	54	51	56	52

^a Average of 30 fruits. ^b Average of 25 fruits.

exposed fruits were taken on several cloudless days, readings being made every two hours between 10 a.m. and 4 p.m.

Bagging the fruits proved to have only slight or no effect on the average fruit weight and diameter (Table 6). When the fruits were covered from the time they were 15 days old (early green stage) until maturity they required 3 to 4 days longer to ripen than the controls. This difference was found to be significant only at the 5 percent level; when the fruits were covered for a shorter period at 25 days after flowering there was no significant delay in maturity (see page 10).

Bagging also caused the disappearance of the green color in the developing fruits, but did not prevent the normal development of red pigment as indicated by pigment analyses.

On cloudless days the temperatures of the bagged fruits were 1 to 2 degrees C. lower than those of fruits exposed to direct sunlight.

Greenhouse Experiments

An attempt was made to determine the effect of foliage on the ascorbic-acid content of the fruits in the greenhouse during the fall of 1948. But when the leaves adjacent to a cluster were removed, the fruits failed to develop satisfactorily, when they developed at all. It was noticed, however, that the fruits on the upper clusters were much higher in ascorbic acid than those on the lower clusters. An experiment was therefore made to determine whether this difference was due to exposure to light or to position on the plant, or to both.

Effect of light and position of clusters. Tomatoes of the Lloyd forcing variety grown in the spring of 1949 were used. Fruits on the experimental plants were thinned to two per cluster. Alternate clusters on a plant were bagged approximately 15 days after flowering. On half the plants the bagged fruits were uppermost and on the other half the exposed fruits were uppermost. Data were taken on the second, third, fourth, and fifth clusters. The first two were considered lower and the second two upper. Fruits of the lower clusters ripened between May 25 and June 27, while those of the upper clusters ripened between June 6 and July 8.

Excluding light from the fruits greatly decreased the ascorbic-acid content (Table 7), 33.1 percent in the upper clusters and 24.6 percent in the lower ones. The previous finding that fruits highest on the plant are also highest in ascorbic acid was confirmed. The upper bagged fruits were higher in ascorbic acid than the lower ones.

Effect of fruits per cluster. Since the number of fruits per cluster on greenhouse tomatoes varies widely, the effect of this variation on

Table 7.—Ascorbic-Acid Content of Bagged and Exposed Tomato Fruits on Different Parts of the Plant

(Data are given in milligrams per 100 grams fresh fruit: average of 16 analyses)

Fruit position and treatment	Ascorbic-acid content Mean—S.E.	Treatments compared			
		Nos.	Difference in ascorbic-acid content	L.S.D.	
				At 5%	At 1%
Upper clusters ^a		Bagged vs. exposed			
1. Bagged.....	25.7 ± 0.51	1-2	12.7	2.29	3.09
2. Exposed.....	38.4 ± 1.00	3-4	7.0	2.71	3.65
Lower clusters ^b		Upper vs. lower clusters			
1. Bagged.....	21.5 ± 1.01	1-3	4.2	2.31	3.11
2. Exposed.....	28.5 ± 0.86	2-4	9.9	2.70	3.63

^a Fourth and fifth clusters. ^b Second and third clusters.

the ascorbic-acid content was studied. Eight plants of the Lloyd forcing variety in the same row receiving about the same light were selected. The fruits on half these plants were thinned to two per cluster. Fruits on the other plants were not thinned. The lower fruits (Clusters 2 and 3) were harvested between May 16 and June 29, the upper fruits (Clusters 4 and 5) between June 4 and July 2.

Thinning to two fruits per cluster caused a significant increase, 10.8 percent, in the ascorbic-acid content of the upper clusters, and a very significant increase, 19.1 percent, in the content of the lower clusters (Table 8).

Table 8.—Ascorbic-Acid Content of Tomato Fruits From Thinned and Unthinned Clusters on Different Parts of the Plant

(Data are given in milligrams per 100 grams fresh fruit)

Fruit position and treatment	Fruits per cluster	Number of fruits analyzed	Ascorbic-acid content Mean—S.E.	Treatments compared			
				Nos.	Difference in ascorbic-acid content	L.S.D.	
						At 5%	At 1%
Upper clusters ^a				Thinned vs. not thinned			
1. Thinned.....	2.0	16	35.7 ± 1.13	1-2....	3.4	3.15	4.21
2. Not thinned.....	3.3	25	32.3 ± 1.07	3-4....	4.6	2.49	3.33
Lower clusters ^b				Upper vs. lower clusters			
3. Thinned.....	2.0	16	28.7 ± 1.00	1-3....	7.0	3.08	4.15
4. Not thinned.....	3.7	26	24.1 ± 0.72	2-4....	8.2	2.59	3.46

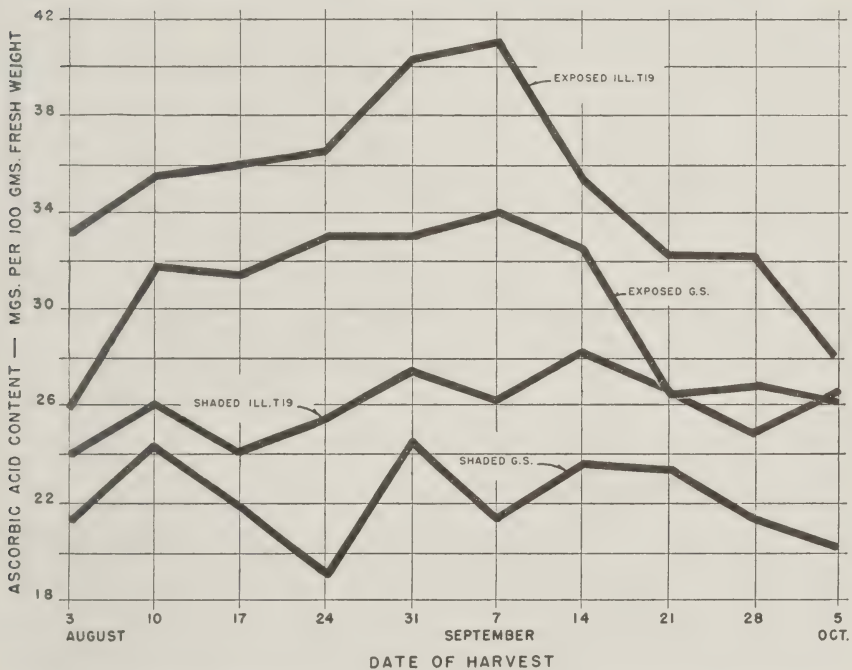
^a Fourth and fifth clusters. ^b Second and third clusters.

Seasonal Variation in Ascorbic-Acid Content

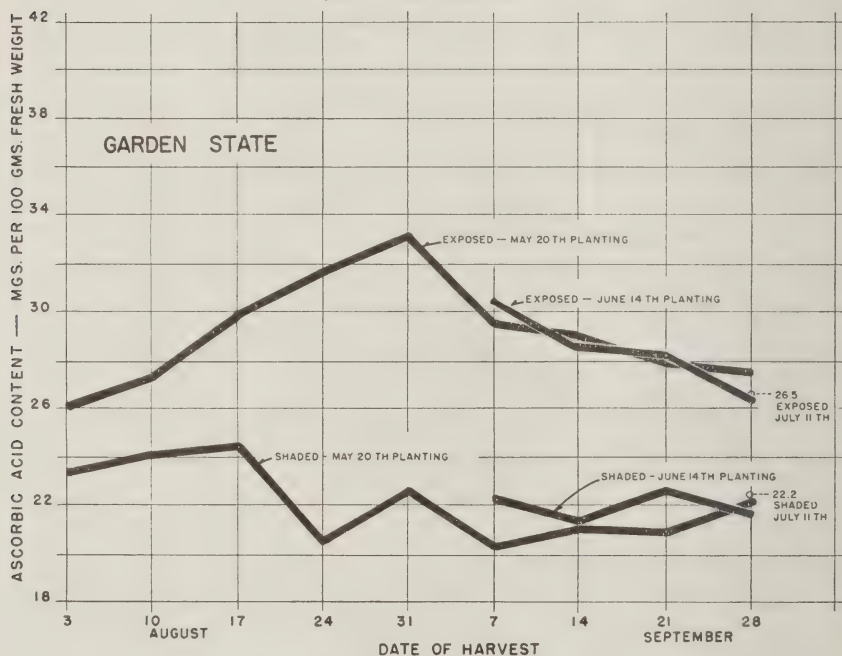
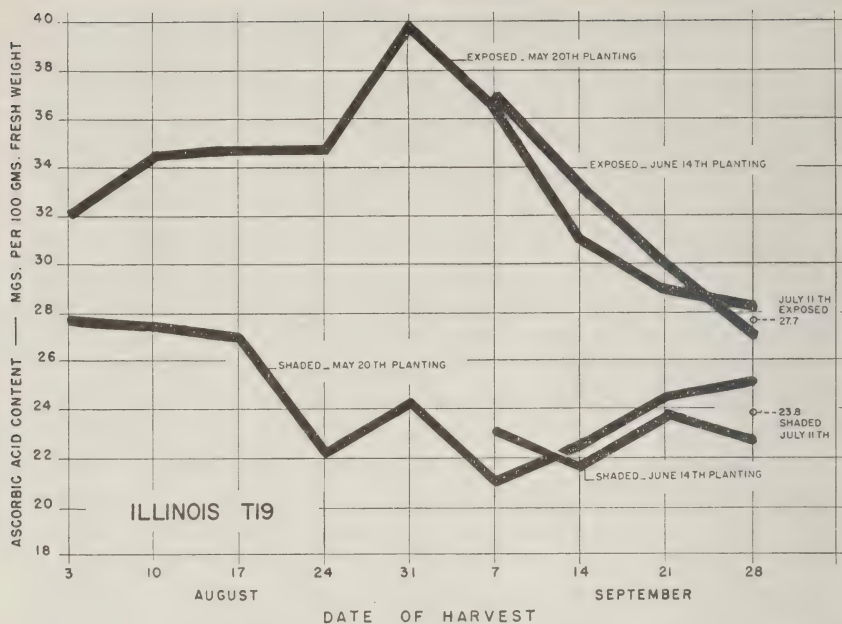
Fruits of Illinois T19 and Garden State were analyzed weekly during the 1948 season for ascorbic-acid content. Samples of 12 to 15 shaded and the same number of exposed fruits from each variety were analyzed separately. Analyses began August 3 and continued to October 5.

The exposed fruits of both varieties proved higher in ascorbic acid than the naturally shaded fruits (Fig. 1), but the former showed greater variation during the season. About the middle of the season the ascorbic-acid content of the exposed fruits reached a peak and thereafter declined sharply. The fruits of Illinois T19 were significantly higher in ascorbic acid than those of Garden State.

The study of seasonal variation in ascorbic acid was repeated during 1949 with the same varieties. Additional treatments were included to find out whether the decrease in ascorbic acid in the exposed fruits at the end of the season might be partly due to deterioration of



During the 1948 season, exposed fruits showed a much higher seasonal variation in ascorbic-acid content than shaded fruits. (Fig. 1)



Physiological condition of the plant resulting from differences in maturity had no effect upon ascorbic-acid content. (Fig. 2)

the plants. Successive plantings were made on May 21, June 14, and July 11. Sampling was done as in the previous season. Harvesting of the first planting began on August 3, the second on September 7, and the third on September 28, when the season ended.

There was the same trend as in the previous season. Again an increase occurred in the ascorbic-acid content of the unshaded fruits until midseason (Fig. 2), followed by a significant decrease toward the end of the season. Fruits with similar light exposures from the three different plantings were found to have about the same ascorbic-acid content at any given time during the season.

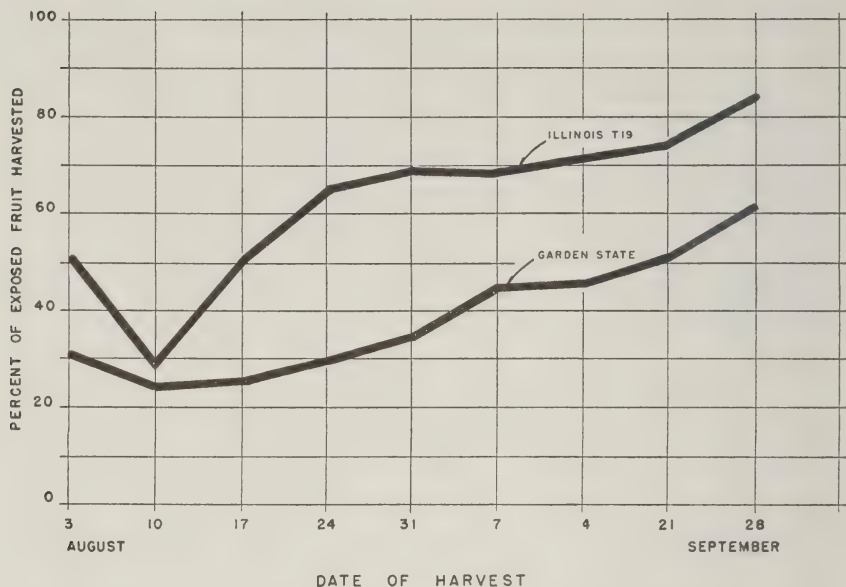
Seasonal Variation in Number of Exposed and Shaded Fruits

The shaded and exposed fruits of Illinois T19 were found to be higher in ascorbic acid than the respective shaded and unshaded fruits of Garden State. The field-run tomatoes of these varieties should also show a difference due to the more dense foliage of Garden State. A study was made therefore to determine the percent of exposed fruits in these varieties during the season. The number of shaded and exposed fruits from 10 plants of each variety were harvested and recorded at weekly intervals throughout the 1949 season.

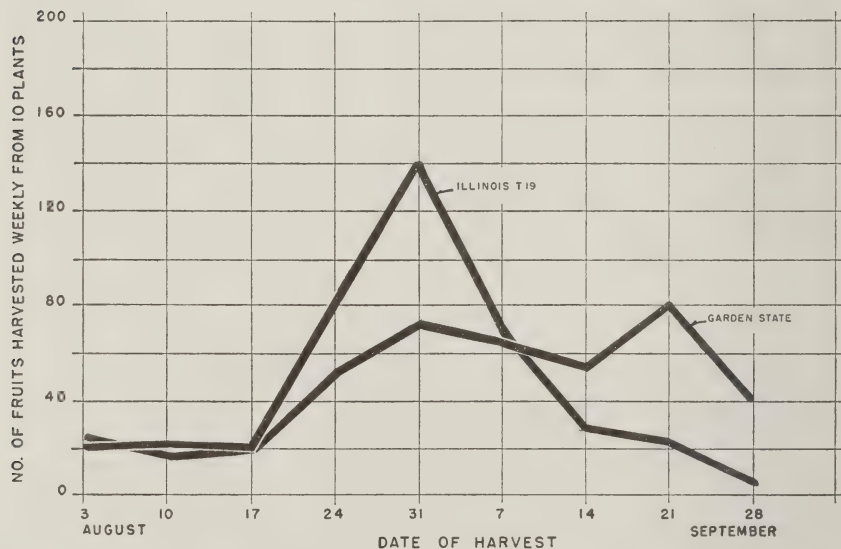
The percentage of exposed fruits harvested from Illinois T19 during the season was 64.6, and from Garden State 41.3. Both varieties, especially Illinois T19, showed a strong initial increase in percent of exposed fruits, then a leveling off, and then a gradual increase to the end of the season (Fig. 3). The total-yield curve of Illinois T19 showed a sharp peak through August 31, followed by a sharp decrease (Fig. 4). Garden State did not show such a sharp peak in yield but continued to produce over a longer period than Illinois T19.

DISCUSSION

From the data presented it is obvious that the exposure of the fruit to sunlight is an important factor affecting the ascorbic-acid content of tomatoes. The percentage increase due to light exposure was higher with Garden State (55.6) than with Illinois T19 (41.4). The Garden State variety has heavier foliage and for this reason its shaded fruits perhaps received less illumination than those of Illinois T19. The percentage difference between the shaded and exposed fruits of Garden State is also higher because of its lower ascorbic-acid content.



The percent of exposed fruit harvested from Illinois T19 during the 1949 season was higher than that from Garden State. For both varieties the percent increased toward the end of the season because of defoliation. (Fig. 3)



T19 showed a higher seasonal peak of production than Garden State. With both varieties the high peak of production coincided with the high peak of ascorbic-acid content (Fig. 2). (Fig. 4)

The data are in agreement with those obtained in earlier tests by McCollum^{24, 25} indicating that sampling error may be greatly reduced by selecting fruits according to light exposure. They also suggest that many of the discrepancies found in the literature concerning the ascorbic-acid content of tomatoes may be due in part to failure to consider light exposures of the fruit. Although the standard error increases with a decrease in number of fruits per sample, it is very significant that when fruits are selected for light exposure, a difference in ascorbic acid of 2 to 3 milligrams per 100 grams of fruit can be measured with a 10-fruit sample.

Fully exposed fruits can be easily selected, but even with careful selection there will be some variation in the light exposure of the shaded fruits. This is indicated by the higher coefficient of variation found in the shaded fruits. The data indicate that more accurate comparisons between varieties may be made by selecting samples from fully exposed, rather than from shaded, fruits. The reverse, however, might have been true if the samples for the test reported in Table 1, page 8, had been taken later in the season after the plants were partially defoliated. It would then have been difficult to determine whether or not an exposed fruit had formerly been shaded.

When developing fruits are enclosed with aluminum-covered paper to exclude light, they develop normally except for the absence of green color. Apparently there is no measurable effect on fruit size, but a small delay in maturity. This is perhaps a result of a slightly lower temperature of the bagged fruits as compared with that of those exposed to direct sunlight. This temperature difference should have no measurable effect on ascorbic-acid content. The increased ascorbic-acid content of exposed compared with bagged fruits may be attributed to solar illumination received by the exposed fruits.

The ascorbic-acid content of tomato fruits appears not to be increased by light exposure during ripening or after the disappearance of chlorophyll from the fruits (Table 2, page 9). The accumulation of ascorbic acid can be assumed, then, to be associated with some aspect of photosynthesis, as suggested by McCollum.²⁵ This is in line with the work of Somers, Kelly, and Hamner,³⁸ who found that ascorbic acid in leaves could not be increased by light in the absence of carbon dioxide.

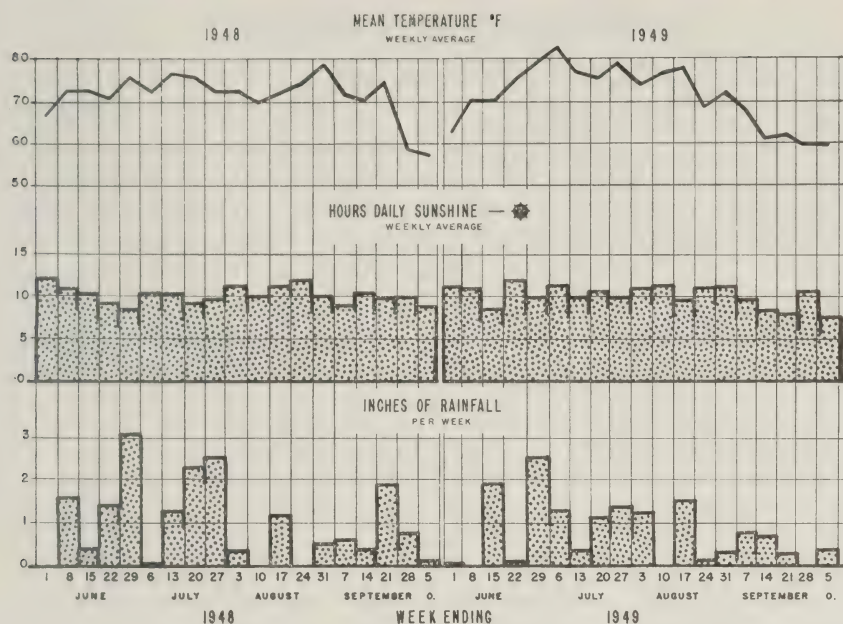
Fruits covered for 10 days early in their development and then exposed to direct sunlight until ripe were lower in ascorbic acid than similar fruits not covered (Table 4, page 11). These data indicate that the effect of sunlight is cumulative, and for a fruit to contain the maximum amount of ascorbic acid, it must be exposed to sunlight through-

out its development. Somers, Hamner, and Kelly³⁹ found no correlation between relative illumination and ascorbic-acid content of tomatoes. Since their samples were apparently not selected for light exposure, and since illumination was recorded for only three weeks prior to harvest, little if any correlation should have been expected. When exposed ripe fruits are used in sampling for ascorbic acid, the probability of foliage coverage earlier in their development should be considered.

The data in Table 5, page 12, show that bagged fruits have about the same ascorbic-acid content on shaded and exposed plants. The same is true for shaded fruits. Ascorbic-acid content closely parallels the amount of illumination reaching the fruit and is only slightly affected by that reaching the leaves under field conditions. Similar results were also shown by Somers, Hamner, and Kelly.³⁹ Even though shading the plants decreases the total solids of the fruits, it apparently has no significant effect on ascorbic acid.

Uppermost fruits on greenhouse plants were found to be highest in ascorbic acid (Table 7, page 14). This could have been due in part to a difference in light exposure, but it was also true for fruits from which light was excluded about 15 days after flowering. Since no seasonal trend could be shown in this experiment, it may be assumed that this difference is due to position on the plant and related to the availability of precursors for the development of ascorbic acid. This is also indicated by the fact that thinning the fruit increases the ascorbic-acid content.

When ascorbic acid was determined at intervals throughout the seasons of 1948 and 1949, weekly variations but no definite trends were found with shaded fruits. However, during the latter season somewhat higher values were obtained with the early fruits. At this time the plants were still producing new foliage which might have shaded some of the sampled fruits only a short time before ripening. These higher values might therefore have been due to illumination during early development. The exposed fruits, on the other hand, showed a very significant peak about the middle of the harvesting season (Figs. 1 and 2). There is no obvious relation between this trend and the weather data (Fig. 5). Since the effect of weather on the ascorbic-acid content of tomatoes is cumulative, a close relationship between weather at any definite time and the ascorbic-acid content of tomatoes is not to be expected. A decrease in ascorbic acid toward the end of the harvesting season might be expected on the basis of plant



Weather conditions during the 1948 and 1949 seasons had no significant effect on the ascorbic-acid content of tomatoes. (Fig. 5)

senility, but fruits from young plants (Fig. 2) showed a trend almost identical with those of old plants. These data preclude the possibility that this trend could have been due largely to the sampling of fruits that were shaded earlier in their development. Differences in ascorbic acid between samples of tomatoes harvested one month apart and attributed to soil type by Hester and Kohman¹⁷ could just as easily have been explained on the basis of seasonal effects.

Tomato strains may vary in time of maturity, making it difficult to sample all for ascorbic acid at one time during the season. When this is the case, shaded fruits should give a more accurate sample than those exposed to direct sunlight.

Not only were the respective shaded and exposed fruits of the Illinois T19 variety higher in ascorbic acid than those of Garden State, but this variety also produced a higher percentage of exposed fruits (Fig. 3) and ripened more of them at a time when ascorbic acid was highest (Fig. 4). In order, then, to determine the tendency of a strain of tomatoes to produce fruits high in ascorbic acid, attention must be given to genetical potential, fruit shading, and the yield curve.

SUMMARY AND CONCLUSIONS

Tomato fruits exposed to direct sunlight were found to be significantly higher in ascorbic acid than shaded fruits. The data indicate that sampling error can be greatly reduced by selecting fruits with regard to light exposure. When a number of treatments are sampled at one time for ascorbic acid, more accurate comparisons can be made by using fruits exposed to direct sunlight. By selecting fruits in this manner, a difference of two to three milligrams of ascorbic acid per 100 grams of fresh fruit can be measured with a 10-fruit sample. During both the 1948 and 1949 season, and with two varieties, exposed fruit reached a peak in ascorbic acid about the middle of the harvesting season and showed greater seasonal variation than shaded fruit. Shaded fruit should therefore be used when strains or treatments are sampled at different times during the season.

The uppermost fruit on greenhouse plants are highest in ascorbic acid. The location of the fruit on the plant therefore is an important factor in sampling for ascorbic-acid content.

Illumination has a cumulative effect on ascorbic acid throughout the green stage of the fruit. The maximum ascorbic-acid content is therefore not reached if a fruit is shaded for any length of time during its development. Ascorbic-acid content varies with the intensity of the illumination reaching the fruits but is apparently not affected by that reaching the leaves under conditions in the field.

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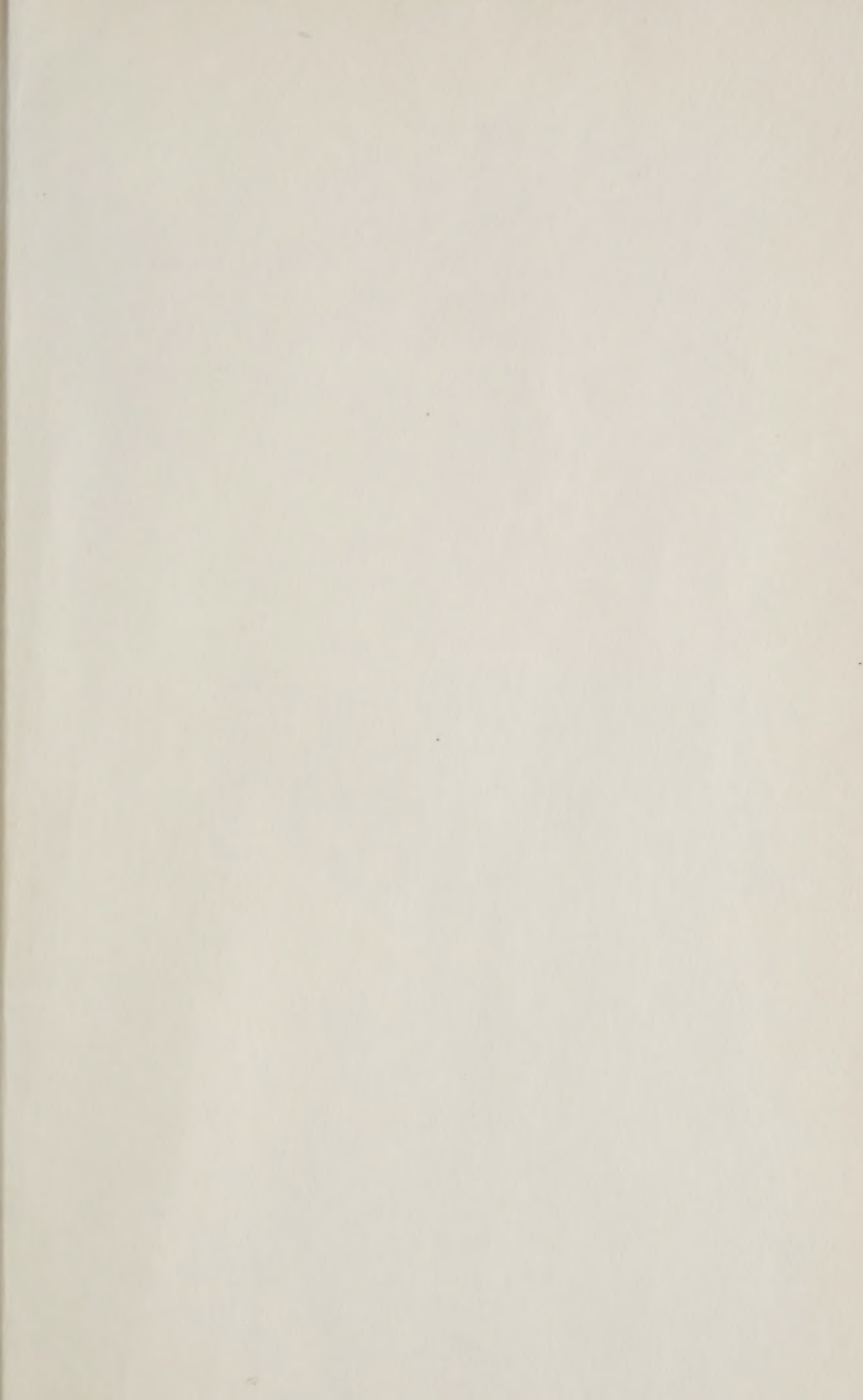
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